REMARKS

This is in response to the Office Action mailed March 16, 2009. Claims 1-20 are pending for consideration. Claims 18-20 have been withdrawn from consideration. Claims 1, 2, 4-8, 16, and 17 are amended herewith. Claims 21 and 22 are added. Support for the new claims can be found in original claims 1 and 2. With entry of this Amendment, claims 1-22 will be pending for consideration.

I. Election of Species

Applicants thank the Examiner for withdrawing the election of species requirement.

II. Objection to the Specification

The Examiner objects to the Abstract. In response, Applicants have amended the Abstract.

III. Claim rejections – 35 USC § 112

Claims 1-17 stand rejected under 35 USC § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Specifically, the Examiner objects to the term "medium" in step d) of claim 1 for lacking an antecedent basis. In response, applicants have amended claim 1 by replacing the expression "*by adding to the medium containing the aforementioned microorganism*" with "*by adding to the sample containing the microorganisms.*"

The Examiner also objects to the recitation of "the transformation of the substrate..." in step d). In order to clearly indicate that the substrate has a fluorescent precursor, claim 1 has been amended to recite that at least one substrate comprises "*one part specific to the enzymatic activity to be indicated and one fluorogenic label.*" This amendment finds support at, for example, page 19, lines 13 to 16, of the

specification, as filed.

The Examiner objects to term "conditioning step" in claims 4 and 7. In response, applicants have amended claims 4 and 7 to reword them and to delete the expression "*conditioning step*" This amendment finds support on page 15, lines 17 to 22 of the patent application as filed, which indicates that "*by "conditioning" . . . it is meant "inducing or activating."*

The Examiner also objects to claim 8 for reciting "*conditioning medium*" and "*medium.*" In response, applicants have amended claim 8 to replace the offending terms with the term "*sample*."

The Examiner objects to claims 5 and 6 for reciting "can." In response, the term "*can*" has been deleted.

The Examiner objects to claim 6, arguing that it is confusing how step c) can take place before step b) or after step d). Applicants respectfully disagree. One of skill in the art would understand from the specification that it would have been possible to carry out step c) before step b) or after step d). Thus, if step c) took place before step b), it would have been obvious for one skilled in the art to induce or activate at least one enzymatic activity of the microorganism after having immunomagnetically concentrating them. In further response, applicants have amended claim 1 to remove any antecedent basis issues and to clarify that the sequence of steps is not critical.

The Examiner has objected to the recitation of "preferably" in claim 17. In response, the term "*preferably*" has been deleted, thus rendering the objection moot.

In claims 2, 7 and 16, the term "*approximately*" has been deleted. Applicants have replaced this term with "about", which is an acceptable term in US patent nomenclature.

The Examiner has objected to the unit µ/L in claim 2. In response, applicants have amended claim 2 to recite the enzymatic unit IU/L. Support can be found in claim

2, as filed. Thus, Applicants respectfully request this objection be withdrawn.

In view of the above amendment and comments, applicants respectfully request the Examiner to reconsider and withdraw all rejections under 35 USC § 112.

IV. Claim rejections – 35 USC § 103

Claims 1, 4, 5, 6, 11, 14, 15 and 17 stand rejected under 35 USC 103(a) as being unpatentable over Berg *et al.* (WO 89/04372, 18th May 1989, "Berg" (cited in IDS)) in view of Pyle *et al.* (WO 95/31481 23 November 1995 "Pyle" (cited in IDS)). Applicants respectfully traverse this rejection.

Berg discloses a method comprising the steps of:

a) selectively enriching the microorganism sought in the sample;

b) inducing at least one enzymatic activity of the aforementioned microorganism;

c) labeling the microorganism; and

d) detecting and analyzing the fluorescence to determine the microorganism concentration.

Berg also states that the claimed process can include a further concentration step by filtration of the microorganisms.

In contrast to the present invention, Berg does not disclose a method comprising a specific step of concentration of the microorganism of interest Additionally, Berg does not disclose a strictly intracellular labeling for purposes of enabling a numeration or counting of the microorganisms, nor a step of numeration or counting of the microorganism. These distinctions are important for the following reasons:

- the specific concentration step enhances the sensitivity of detection by a factor

of 10 (see page 18, lines 9 to 14 of the specification as filed);

- the strictly intracellular labeling permits a direct and quick numeration or counting of microorganisms. Berg uses a counting method that requires many steps. Specifically, Berg uses permeability agents which necessitates many measures and then requires a comparison with a standard curve to determine the concentration of microorganisms in the sample; and

- the enumeration or counting of the microorganisms by a technique chosen from the group comprising flow cytometry, filtration cytometry and fluorescence microscopy is fast and reliable.

Thus, the technical effects associated with these differences permits an accelerated microorganism detection, in less than 24 hours for some of them, with the detection being carried out with a better sensitivity and specificity than the one obtained according to the methods described in Berg. The benefits of applicants invention relative to the prior art are described in the specification at page 13.

The Examiner has cited Pyle for its teaching of a method for detection and enumeration of viable microorganisms in a sample, which relies on a metabolic indicator and uses immunomagnetic separation and concentration using antibodies that specifically bind to a target bacteria. According to the Examiner, it would have been *prima facie* obvious to immunomagnetically concentrate the enzymatically induced microorganisms of Berg, in view of the teachings of Pyle.

Applicants argue that Pyle does not remedy the deficiencies of Berg, which does not describe, nor suggest, the important specificity and sensitivity obtained with the claimed method, nor the reduced time needed to carry out this method.

Accordingly, Applicants respectfully submit that pending claim 1 and the claims which depend from claim 1 are new and inventive in view of Berg and Pyle.

Claim 16 stands rejected under 35 USC 103(a) as being unpatentable over Berg

et al. (WO 89/04372, 18th May 1989 (cited in IDS)) and Pyle *et al.* (WO 95/31481 23 November 1995 (cited in IDS)) as applied to claims 1, 4, 5, 6, 11, 14, 15 and 17 above, further in view of Sigma catalog 1996 p. 2179-2181. Applicants respectfully traverse this rejection. Berg and Pyle have been discussed above. The Examiner cites the Sigma catalog for showing the availability of filters with various porosity sizes, including between 20 and 25 and 30 microns. Applicants traverse this rejection for reasons set forth above in connection with the rejection over Berg and Pyle. Applicants agree that filters of various porosity were available at the time of the invention. However, nothing in the cited art directs the skilled artisan to porosity sizes within the claimed ranges. As such, applicants do not agree that the subject matter of claim 16 would have been obvious to the skilled artisan. Withdrawal of this rejection is respectfully requested.

Claims 8, 9 and 10 stand rejected under 35 USC 103(a) as being unpatentable over Berg *et al.* (WO 89/04372, 18th May 1989 (cited in IDS)) and Pyle *et al.* (WO 95/31481 23 November 1995 (cited in IDS)) as applied to claims 1, 4, 5, 6, 11, 14, 15 and 17 above, further in view of Olsen *et al.* Plant and Soil 186:75-79, 1996.

Applicants respectfully traverse this rejection for reasons set forth above with regard to the teachings of Berg and Pyle. Olsen teaches an immunomagnetic method used for separating bacteria from a sample. However, it does not teach or suggest the other steps recited in independent claim 1 so it does not remedy the deficiencies in Berg and Pyle. Accordingly, applicants respectfully request the Examiner to reconsider and withdraw this rejection.

Claims 12 and 13 stand rejected under 35 USC 103(a) as being unpatentable over Berg *et al.* (WO 89/04372, 18th May 1989 (cited in IDS)) and Pyle *et al.* (WO 95/31481 23 November 1995 (cited in IDS)) as applied to claims 1, 4, 5, 6, 11, 14, 15 and 17 above, further in view of Boyd *et al.* US 5,510,243 April 23, 1996. According to the Examiner, Boyd teaches using a xanthene label part and a monosaccharide on a substrate. The Examiner further opines that it would have been obvious to substitute one label and substrate for another with an expectation of success.

Applicants respectfully traverse this rejection for the reason set forth above in connection with Berg and Pyle. Boyd fails to remedy the deficiencies in the primary references. Accordingly, applicants respectfully request the Examiner to reconsider and withdraw the rejection of claims 12 and 13.

Claim 7 stands rejected under 35 USC 103(a) as being unpatentable over Berg *et al.* (WO 89/04372, 18th May 1989 (cited in IDS)) and Pyle *et al.* (WO 95/31481 23 November 1995 (cited in IDS)) as applied to claims 1, 4, 5, 6, 11, 14, 15 and 17 above, further in view of Kaclikova *et al.* Journal of Microbiological Methods, Vol. 46, Issue 1, July 2001, p. 63-67. According to the Examiner, Kaclikova teaches a method of detecting *Listeria* in which the pathogens are enriched with a Half-Fraser broth, which is said to contain yeast extract. Applicants respectfully traverse this rejection. First, Kaclikova teaches against the use of using a half-Frasier enrichment broth, stating that this method had significant drawbacks associated with time-consuming cultures (page 63). The point of Kaclikova's work was to develop an improvement over the prior half-Frasier/Frasier methods. In any event, nothing in Kaclikova remedies the deficiencies in the primary references. Accordingly, applicants respectfully request the Examiner to reconsider and withdraw the rejection over claim 7.

Applicants understand that the Examiner considers claim 2 to be free of the prior art. Accordingly, new claims 21 and 22 also should be considered free of the prior art and allowable. However, in view of the above arguments, applicants respectfully request the Examiner to reconsider and withdraw all the pending obviousness rejections.

CONCLUSION

Should the Examiner believe that anything further is necessary in order to place this application in better condition for allowance, the Examiner is requested to contact the undersigned at the telephone number listed below.

In the event that an extension of time is necessary to prevent abandonment of this application, then such extension of time is hereby petitioned under 37 C.F.R. § 1.136(a), and any fees required therefore are hereby authorized to be charged to our Deposit Account No. 01-2300 referencing docket number 029440.00009.

Respectfully submitted,

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ABSTRACT

A method for detecting and counting the microorganisms in a sample is described. The method comprises:

a) selectively enriching the microorganism sought in the sample,

b) inducing or activating at least one enzymatic activity of the microorganism,

c) immunomagnetically concentrating the microorganism,

d) fluorescently labeling the microorganism, and

e) detecting and analyzing the fluorescence making possible the numeration or counting of the microorganisms sought by flow cytometry, filtration cytometry or fluorescence microscopy.